

VW
H/15/08 10766134

Appl. No. NOT YET ASSIGNED
Amdt. dated January 27, 2004
Preliminary Amendment

PATENT

presumed to be zebrafish cells, were also present in the HepG2 cell masses. There was no evidence of vacuolated space in any of the cell masses, which would suggest that the transplanted cells were dying.

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H/15/08 24
Please replace the paragraph beginning at page 25, line 15 with the following rewritten paragraph:

To confirm that the transplanted HepG2 cells were viable, we examined xenograft embryos for the presence of human VEGF (hVEGF) and human AFP (hAFP), two proteins normally secreted by HepG2 cells in culture and to be present in the blood of patients diagnosed with hepatocellular carcinoma (Huber, 1985; Eriser et al. 1998; Louha et al., 1997). High levels of hVEGF have also been correlated with adverse effects on heart development in zebrafish and other vertebrates (Drake, et al., 1995; Feucht, et al., 1997, Serbedzija, 1999). For these experiments, xenograft embryos were stained with human specific antibodies to either hVEGF or hAFP and these antibodies were detected using RPE labeled secondary antibodies. Because the fluorescence spectra of DiI and RPE are similar, transplantations were performed using unlabeled HepG2 cells. Embryos were collected 24 to 72 hours after transplantation. Transplanted cells exhibit appropriate cell characteristics including the production of proteins. 100% of the embryos stained for VEGF (100) contained labelled cells in cell masses. In addition, in 50% of hVEGF positive embryos, individual RPE-labeled cells were detected in close proximity to the cell mass. As was shown with hVEGF staining, 100% of the embryos stained for hAFP had labeled cells in the cell masses (Figure 5 C and D). In contrast to hVEGF staining, in hAFP positive embryos, no cells were observed outside the cell mass, regardless of when the embryos were collected. For both hVEGF and hAFP antibodies, staining was restricted to HepG2 cells. Neither hVEGF nor hAFP label was observed in non-xenograft control embryos. Claim 61 was canceled.